2A-Adrenergic receptor polymorphisms and mRNA expression levels are associated with delay discounting in cocaine users

Havranek, Michael M ; Hulka, Lea M ; Tasiudi, Eve ; Eisenegger, Christoph ; Vonmoos, Matthias ; Preller, Katrin H ; Mössner, Rainald ; Baumgartner, Markus R ; Seifritz, Erich ; Grünblatt, Edna ; Quednow, Boris B

Abstract: Cocaine users characteristically display preferences for smaller immediate rewards over larger delayed rewards, and this delay discounting (DD) has been proposed as an endophenotype of cocaine addiction. Recent evidence suggests that the norepinephrine system and more specifically the 2A-adrenergic receptor (ADRA2A) are impacted by chronic cocaine use while also being potentially involved in the neural mechanisms underlying DD. Hence, we investigated the effects of ADRA2A polymorphisms and ADRA2A mRNA expression levels on DD of cocaine users and stimulant-naïve controls. Two hundred and twenty-three participants (129 cocaine users and 94 stimulant-naïve healthy controls) completed a computerized DD paradigm and were genotyped for three single nucleotide polymorphisms (SNPs; rs1800544, rs521674 and rs602618) in the ADRA2A gene, while their peripheral ADRA2A mRNA expression was quantified in whole blood samples. The three SNPs were in near-perfect linkage disequilibrium. Accordingly, significant group*genotype interactions were found for all three ADRA2A variants revealing steeper DD in cocaine users (but not in controls) carrying the G-allele of SNP rs1800544, the T-allele of rs521674 and the C-allele of rs602618. Similarly, high ADRA2A mRNA expression levels were significantly associated with a reduced tendency to choose smaller more immediate rewards (over larger delayed rewards) in cocaine users but not in controls. As the relationship between DD and cocaine use was moderated by ADRA2A SNPs and by peripheral ADRA2A gene expression, we propose that the norepinephrine system is involved in DD deficits observed in cocaine using individuals. Consequently, pharmacological compounds targeting ADRA2As might be considered for the symptom-specific treatment of delay aversion in stimulant addiction.

DOI: https://doi.org/10.1111/adb.12324

α_{2A}-adrenergic receptor polymorphisms and mRNA expression levels are associated with delay discounting in cocaine users

Michael M Havranek[a,*], MD; Lea M Hulka[a], PhD; Eve Tasiudi[b], MSc; Christoph Eisenegger[c], PhD; Matthias Vonmoos[a], PhD; Katrin H Preller[a], PhD; Rainald Mössner[d], MD; Markus R. Baumgartner[e], PhD; Erich Seifritz[a,f], MD; Edna Grünblatt[b,f], PhD; Boris B Quednow[a,f,*], PhD

[a]*Department of Psychiatry, Psychotherapy and Psychosomatics, Psychiatric Hospital, University of Zurich, Switzerland*
[b]*University Clinic for Child and Adolescent Psychiatry, University of Zurich, Switzerland*
[c]*Neuropsychopharmacology and Biopsychology Unit, Institute of Basic Psychological Research and Research Methods, University of Vienna, Austria*
[d]*Department of Psychiatry and Psychotherapy, University of Tübingen, Germany*
[e]*Center of Forensic Hairanalytics, Institute of Legal Medicine, University of Zurich, Switzerland*
[f]*Neuroscience Center Zurich, University and ETH Zurich, Switzerland*

Abbreviated Running Title
ADRA2A and reward impulsivity

Manuscript Category
Original Article

Revision resubmitted
August the 19th, 2015

Manuscript Characteristics
Number of words in the abstract: 250
Number of words in the article: 3723
Number of figures / tables: 2 / 2
Supplementary Information: yes

Conflict of Interest
None of the authors declared any conflict of interest.

***Corresponding authors:**
Boris B. Quednow, PhD; Michael M. Havranek, MD
Experimental and Clinical Pharmacopsychology
Psychiatric Hospital of the University of Zurich
Lenggstrasse 31, CH-8032 Zurich, Switzerland
Tel.: 0041-44-384-2777 / Fax: 0041-44-384-3396
E-Mail: quednow@bli.uzh.ch, m.havranek@bli.uzh.ch

Abstract

Cocaine users characteristically display preferences for smaller immediate rewards over larger delayed rewards and this delay discounting (DD) has been proposed as an endophenotype of cocaine addiction. Recent evidence suggests that the norepinephrine system and more specifically the α_{2A}-adrenergic receptor (*ADRA2A*) are impacted by chronic cocaine use, while also being potentially involved in the neural mechanisms underlying DD. Hence, we investigated the effects of *ADRA2A* polymorphisms and *ADRA2A* mRNA expression levels on DD of cocaine users and stimulant-naïve controls. Two-hundred-twenty-three participants (129 cocaine users, 94 stimulant-naïve healthy controls) completed a computerized DD paradigm and were genotyped for three single nucleotide polymorphisms (SNPs; rs1800544, rs521674, and rs602618) in the *ADRA2A* gene, whilst their peripheral *ADRA2A* mRNA expression was quantified in whole blood samples. The three SNPs were in near perfect linkage disequilibrium. Accordingly, significant group*genotype interactions were found for all three *ADRA2A* variants revealing steeper DD in cocaine users (but not in controls) carrying the G-allele of SNP rs1800544, the T-allele of rs521674, and the C-allele of rs602618. Similarly, high *ADRA2A* mRNA expression levels were significantly associated with a reduced tendency to choose smaller more immediate rewards (over larger delayed rewards) in cocaine users but not in controls. As the relationship between DD and cocaine use was moderated by ADRA2A SNPs and by peripheral ADRA2A gene expression, we propose that the norepinephrine system is involved in DD deficits observed in cocaine using individuals. Consequently, pharmacological compounds targeting ADRA2As might be considered for the symptom-specific treatment of delay aversion in stimulant addiction.

Keywords: delay of gratification, intertemporal choice, drug dependence, impulsivity, mutation, self-control, environment, noradrenaline

Introduction

Maladaptive decision-making has been suggested as a core feature in stimulant addiction as cocaine users characteristically seek the instantaneous and brief reinforcement of the drug despite its negative future consequences (Hulka et al., 2014; Koob, 2009; MacKillop, 2013). At the basis of this behavior lies an impulsive preference for smaller immediate rewards over larger delayed rewards coined as delay discounting (DD). Several studies provided evidence that cocaine users display higher discounting rates for future rewards compared to stimulant-naïve controls (Bickel et al., 2011; Coffey et al., 2003; Heil et al., 2006; Hulka et al., 2014; Kirby and Petry, 2004). In addition, stronger DD has not only been associated with higher levels of drug consumption and poor treatment response in cocaine users but also with general negative outcomes in financial, professional, and health domains (Brody et al., 2014; Mischel et al., 2011; Moffitt et al., 2011; Washio et al., 2011). However, despite the importance of DD in cocaine addiction, its molecular underpinnings remain unclear so far.

Evidence from human and animal studies suggests the involvement of the norepinephrine (NE) system and more specifically the α_{2A}-adrenergic receptor (*ADRA2A*) in impulsive decision-making in general and in DD in particular. Studies performed in rats and in humans revealed that the NE reuptake inhibitor atomoxetine improved performance and decreased premature responding in stop-signal reaction time tasks (as an example for motor impulsivity) (Bari and Robbins, 2013; Robinson et al., 2008). An experiment in rhesus monkeys has shown that the *ADRA2A* agonist guanfacine selectively reduced the animals' preference to choose smaller more immediate rewards (over larger delayed rewards) without affecting their risk preference (Kim et al., 2012). While the presynaptic function of the *ADRA2A* is to inhibit NE transmitter release (suppression of NE release by negative feedback), it is also the most prevalent postsynaptic NE receptor in the prefrontal cortex (PFC) (Arnsten et al., 1996). Thus, it has been proposed that an activation of ADRA2As in the PFC might be the mechanism by which guanfacine

decrases DD (Kim et al., 2012). Moreover, several genetic studies support an association of the G-allele of the *ADRA2A* C-1291G (rs1800544) single nucleotide polymorphism (SNP) with attention deficit hyperactivity disorder (ADHD) (Park et al., 2005; Roman et al., 2006; Schmitz et al., 2006). Accordingly, ADHD has also been associated with a preference to choose immediate over delayed rewards (Noreika et al., 2013) as well as with an increased vulnerability for addictive disorders (Urcelay and Dalley, 2012). Moreover, the G-allele of the ADRA2A rs1800544 SNP has been associated with higher vascular reactivity to cold and psychosocial stress corroborating the functional relevance of this SNP (Kelsey et al., 2012).

Acute cocaine administration affects the NE system by inhibiting NE reuptake at the respective transporters and thus increasing synaptic NE signaling (Clark et al., 1991; Fitzgerald, 2013; Levy and Blattberg, 1978; O'Neill et al., 2013). However, it is less clear how chronic cocaine use affects NE signaling. Recent evidence suggests that chronic use modifies the NE system by mechanisms involving ADRA2As (Fitzgerald, 2013). For example, rats exposed to seven days of cocaine injections displayed permanent desensitization of ADRA2As (measured by a blunted growth hormone response to clonidine challenges) (Baumann et al., 2004). Furthermore, the above mentioned *ADRA2A* agonist guanfacine has been associated with lower cocaine craving, anxiety, and arousal in patients recovering from cocaine dependence, whereas the *ADRA2A* antagonist yohimbine facilitated reinstatement of cocaine seeking during early withdrawal in rats (Buffalari et al., 2012; Fox et al., 2012).

Taken together, the NE system and in particular the *ADRA2A* are implicated in both DD and in the neurochemical adaptations after chronic cocaine consumption. Thus, our goal was to investigate whether three *ADRA2A* SNPs are associated with DD in chronic cocaine users compared to stimulant-naïve healthy controls. In addition to the afore-mentioned rs1800544, we further examined *ADRA2A* rs521674 and rs602618, which have been shown to be in linkage disequilibrium (LD) with rs1800544 in European and Asian populations (Clarke et al., 2012; Li et al., 2012). As specific radioligands for molecular

imaging of the *ADRA2A* are still lacking, we determined ADRA2A mRNA expression levels in peripheral blood in order to clarify the functional significance of the investigated polymorphisms. We hypothesized that both the *ADRA2A* polymorphisms and the *ADRA2A* mRNA expression levels are associated with DD preferences in chronic cocaine users.

Materials and Methods

Participants

A total sample of 223 participants (129 chronic cocaine users and 94 stimulant-naïve controls) was investigated as part of the longitudinal Zurich Cocaine Cognition Study (ZuCo2St: Hulka et al., 2014; Preller et al., 2014; Vonmoos et al., 2013; Vonmoos et al., 2014). Cocaine users were included if they indicated cocaine as their primary drug of choice, showed a use of >0.5g/month, and a maximum abstinence duration no longer than 6 months. Exclusion criteria for all participants were any current or previous neurological disorders or head injuries and use of prescription drugs affecting cognitive functioning. Additional exclusion criteria for the cocaine users were regular use of opioids or a polytoxic drug use pattern according to DSM-IV as well as any current or previous Axis-I DSM-IV psychiatric disorders with exception of cocaine and alcohol abuse/dependence, a history of depression, and ADHD. Specific exclusion criteria for the control group were regular drug use (lifetime >15 occasions) with the exception of nicotine dependence and occasional cannabis use and any current or previous Axis-I DSM-IV psychiatric disorder (American Psychiatric Association, 1994). Participant's self-reports regarding drug use were confirmed by toxicological analyses of urine and hair samples (Table S1). Our sample of 129 cocaine users consisted of 94 regular recreational users (72.8%) and 35 dependent users (27.1%) according to DSM-IV. More detailed information about our sample and about selection, recruitment, and drug screening procedures have been presented in detail in our previous work (Hulka et al., 2014; Preller et al., 2014; Vonmoos et al., 2013). The presented study has been carried out in accordance with the Declaration of Helsinki and was approved by the Cantonal Ethics Committee of Zurich. All participants provided written informed consent prior to the study and were financially compensated for their participation.

Procedure

All participants were examined by trained psychologists using a Structural Clinical Interview (SCID-I) (Wittchen et al., 1997) according to DSM-IV. Drug use was assessed by using a structured and standardized Interview for Psychotropic Drug Consumption (Quednow et al., 2004). The pre-morbid verbal intelligence quotient (IQ) was measured by the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B; multiple choice vocabulary intelligence test) (Lehrl, 1999). Because psychiatric comorbidities such as depression and ADHD are common among cocaine using populations (Perez de Los Cobos et al., 2011; Rounsaville, 2004), we applied the ADHD-Self Rating Scale (ADHD-SR) (Rosler et al., 2004) and the Beck Depression Inventory (BDI) (Beck et al., 1961). In the frame of a comprehensive neuropsychological test battery, participants completed a computerized version of a DD paradigm of the Kirby Monetary Choice Questionnaire *(Kirby and Petry, 2004)* using hypothetical rewards and fixed choices and enabling the calculation of the discounting rate of delayed rewards according to the formula $V=A/(1+ kD)$. Hereby, V is the present value of the delayed reward A at delay D, and k is a free parameter that determines the discounting rate (the larger the parameter k, the stronger the discounting of delayed rewards) (Mazur, 1987). In order to better illustrate comparisons between cocaine users and controls, the main parameter k was z-transformed (based on mean and standard deviation of the control group) and multiplied by -1 so that higher z-scores indicate lower levels of reward impulsivity (i.e., little discounting of delayed rewards).

Genotyping and mRNA Quantification

The *ADRA2A* polymorphisms *(*rs1800544, rs521674, and rs602618) were determined as described in the Supplementary Information (SI). In order to compare G-allele carriers in rs1800544 with non-carriers, the rare GG genotype (8.8%) was pooled with the CG genotype resulting in two genotype groups: CC vs. CG/GG. Similarly, the rare TT genotype (8.8%) of rs521674 was pooled with the AT genotype and the rare CC genotype (9.2%) of rs602618 was pooled with the AC genotype, respectively, resulting in the two

genotype groups: AA vs. AT/TT and AA vs. AC/CC. For the haplotype analysis (provided in the SI) of the three SNPs (rs1800544, rs521674, and rs602618) contrasting non-carrier vs. carriers of minor alleles (CC-AA-AA vs. CG/GG-AT/TT-AC/CC), ten participants with rare allele combinations were excluded.

Total RNA was isolated from whole blood using the RNA isolation NucleoSpin RNA Blood in combination with the NucleoSpin RNA/DNA Buffer Set according to the manufacturer's recommendation (Macherey-Nagel AG, Oersingen, Switzerland). Total RNA samples were spectrophotometrically scanned (NanoVue, GE Healthcare Life Sciences) to obtain the A260/A280 of >1.9 and concentration levels. Additionally, for RNA quality resulting with RIN (RNA integrity number) values, all samples were measured on the automated electrophoresis system (Experion, BioRad Co., Hercules, CA, USA). Quantitative real-time RT-PCR was conducted for *ADRA2A* and six additional reference genes (*ACTB, GAPDH, ALAS1, RPL13A, PPIA,* and 18S ribosomal RNA) as described previously (Grunblatt et al., 2009). Total RNA (500 ng) from each sample was reverse transcribed using iScript cDNA synthesis kit (BioRad Co., Hercules, CA, USA). Each amplification was performed in a total volume of 20µl containing 5µl QuantiTect SYBR Green PCR kit (Qiagen) and the specific primer mix (PrimerAssay- Qiagen). PCR conditions were run according to manufacturer's manual (Qiagen). A melting point analysis was conducted for each assay to confirm specificity of PCR products and all PCR reactions were run in triplicates. The program LinRegPCR (www.hartfaalcentrum.nl) was used to determine the PCR efficiency. Gene expression and normalization analysis with the most stable reference genes was conducted using the QBase plus software (Biogazelle) (Vandesompele et al., 2002). The software detected that the reference gene *RPL13A* was least stable and therefore this gene was excluded and normalization analysis was conducted using the five other reference genes.

Statistical Analyses

Gene effects on DD were analyzed separately for each SNP using analyses of covariance (ANCOVA) with the factors *group* (twofold, cocaine users vs. controls) and *genotype* (twofold, CC vs. CG/GG) and the

covariates *age* and *verbal IQ*. Another ANCOVA with the additional covariates *BDI score, ADHD-SR score,* and *cumulative cocaine dose* was calculated to investigate possible influences of these additional factors. *ADRA2A mRNA expression levels* were stratified into high vs. low mRNA expression using median split. DD was then investigated using ANCOVAs with the factors *group* (as above) and *mRNA expression levels* (high vs. low) and the covariates *age* and *verbal IQ*. Finally, *post hoc t*-tests were conducted on the basis of significant ANCOVA main effects where necessary. Results were considered significant if $p<.05$ after correction for multiple comparisons using the Hochberg method. Hochberg's procedure (a step-up modification of the Bonferroni method) tests each partition hypothesis using all the order statistics by formulating a sequence of critical values based on Simes' inequality (Hochberg, 1988). Statistical analyses were conducted with SPSS (Version 20.0), testing of linkage disequilibrium (LD) between polymorphisms was performed with the software *Haploview* (Barrett et al., 2005) and associations between polymorphisms and cocaine use was analyzed with the Armitage's Trend Test.

Results

Demography and association analysis

The comparison between cocaine users and controls did not reveal significant differences regarding age and sex distribution (Table 1). However, cocaine users had significantly fewer years of education, lower IQ scores, higher scores in the BDI, and higher ADHD-SR scores than controls. Beyond these group differences, one-way ANCOVAs with age and IQ as covariates showed no significant *genotype* (rs1800544) or *group*genotype* interaction effects on BDI (genotype: $F(1,215)=0.40$, $p=.53$; interaction: $F(1,215)=0.03$, $p=.87$) or ADHD-SR scores (genotype: $F(1,215)=0.82$, $p=.37$; interaction: $F(1,215)=0.03$, $p=.85$). For information on the substance use of both groups see Table S1 in the SI. Genotype frequencies of all three polymorphisms were distributed in accordance to the Hardy-Weinberg Equilibrium (Table S2) and all three SNPs were found to be in near perfect linkage disequilibrium (LD) with each other (rs1800544/rs521674: D'=1.0, LOD=92.24, $r^2=0.99$; rs1800544/rs602618: D'=0.98, LOD=79.62, $r^2=0.92$; rs521674/rs602618: D'=0.97, LOD=77.66, $r^2=0.91$)(Figure S1). None of the polymorphisms was associated with cocaine use per se (Table S2) and within the group of cocaine users, genotype groups did not differ regarding cocaine consumption (Table S3).

ADRA2A polymorphisms and DD

Results are reported for rs1800544, however, the same results have been found for the two other SNPs due to the strong linkage disequilibrium (Table S2 and Figure S1). As shown previously in a part of the present sample (Hulka et al., 2014), cocaine users were more likely to choose immediate smaller rewards over larger delayed rewards as indicated by significant *group* effects on DD in small, medium, and large reward magnitudes, respectively (Table 2). There was no significant main effect of the factor *genotype* but a significant *group*genotype* interaction was found across all reward magnitudes (Table 2), reflecting significantly steeper DD in cocaine users carrying the G-allele (rs1800544, across all rewards: $F(1,215)=10.05$, $p_{cor}<.01$, Cohen's $f=0.22$) compared to CG/GG carriers (post hoc test of mean DD across

reward magnitudes: $t(127)=2.93$, $p<.01$, $d=0.56$)(Figure 1, Figure S2). In contrast, controls showed the reverse pattern but the post hoc test did not show a significant difference ($t(90)=-1.45$, $p=.15$, $d=0.33$). This interaction remained significant even when *BDI score, ADHD-SR score,* and *cumulative cocaine dose* were introduced together as additional covariates (rs1800544: $F(1,211)=8.93$, $p_{cor}<.01$, $\eta p^2=0.04$, Cohen's $f=0.21$). Of the covariates' main effects, none was strong enough to survive multiple comparison correction (age: $p_{cor}=.06$; verbal IQ: $p_{cor}=.57$; BDI score: $p_{cor}=.10$; ADHD-SR: $p_{cor}=.98$; cumulative cocaine dose: $p_{cor}=.08$). Analyses of the unpooled rs1800544 genotype (i.e., CC vs. CG vs. GG; see Table S4 and Figure S3) and a haplotype analysis (CC-AA-AA vs. CG/GG-AT/TT-AC/CC; see Table S5 and Figure S4) revealed similar results.

***ADRA2A* mRNA expression and DD**

After mRNA quality control, 104 samples were eligible for the *ADRA2A* gene expression analysis. Peripheral mRNA expression did not differ between cocaine users ($n=54$) and controls ($n=50$) and between genotype groups (Table S6). However, using a median split of the mRNA expression (high vs. low) together with the factor *group* in a two-way ANCOVA revealed a significant *group*mRNA expression* interaction on mean DD across rewards ($F(1,98)=5.22$, $p<.05$, $\eta p^2=0.05$, Cohen's $f=0.23$, Figure 2), mirroring the *group*genotype* interaction shown above (Figure 1). The interaction was explained by more pronounced DD in cocaine users with low levels of *ADRA2A* mRNA expression compared to users with high levels, as indicated by a strong statistical trend and a considerable effect size in the post hoc test ($t(52)=1.83$, $p=.07$, $d=0.50$). Again, controls showed the reverse pattern, which was not significant ($t(48)=-1.53$, $p=.13$, $d=.43$).

Importantly, within the cocaine user group, the period of abstinence of cocaine use (see Table S1) was neither correlated with ADRA2A mRNA expression (Pearson's product moment correlation: r=-.12, p=.36, n=54) nor with DD (r=.05, p=.59, n=129). Also a positive urine test for cocaine (see Table S1) did not affect ADRA2A mRNA expression level in cocaine users (negative: 1.33 +/-1.0 SD; positive 1.19 +/-0.8 SD;

t(51)=.44, p=.66). Moreover, we have previously shown in an overlapping sample, that a cocaine positive urine test does not affect DD (Hulka et al., 2014). Accordingly, inclusion of abstinence duration or cocaine urine screening status as additional covariates in the analyses did not change our results.

Discussion

Examining the molecular mechanisms of DD in cocaine users and healthy controls, we were able to demonstrate that three linked *ADRA2A* SNPs (rs1800544, rs521675, and rs602618) as well as peripheral *ADRA2A* mRNA expression levels mediate reward impulsivity in dependence of cocaine use of the participants. More specifically, we found that in cocaine users, the G-allele of rs1800544 (and the T-allele of rs521674, as well as the C-allele of rs602618) as well as low *ADRA2A* mRNA concentrations were associated with steeper discounting of delayed rewards, while there were no significant differences between genotypes in healthy controls.

Interestingly, the current findings are in line with previous reports on the role of *ADRA2A* polymorphisms in ADHD, as the G-allele of rs1800544 has repeatedly been proposed as a risk allele for ADHD as well as for its symptoms of impulsive decision-making (Gizer et al., 2009; Park et al., 2005; Roman et al., 2006; Roman et al., 2003; Schmitz et al., 2006; Stevenson et al., 2005). However, a large meta-analysis found no consistent evidence for a relationship between the G-allele and ADHD but a significant heterogeneity of results from different studies (Gizer et al., 2009). Consequently, the authors suggested that future studies should explore potential moderators as explanation for this heterogeneity. Accordingly, a recent study on the relationship between *ADRA2A* SNPs (rs1800544, rs553668, and rs1800545) and ADHD found mediating effects of personality (namely associations with novelty seeking, persistence, and harm avoidance) (de Cerqueira et al., 2011). In addition, another study presenting an association of rs1800544 with intra-individual variability in response time suggested that *ADRA2A* SNPs are rather associated with objectively measureable endophenotypes of ADHD (such as intra-individual variability in response time) than with the complete clinical phenotype of ADHD itself (Cummins et al., 2014). In line with these two studies, we provide evidence for an interaction between *ADRA2A* SNPs and cocaine use regarding DD, which has been proposed as an addiction endophenotype (MacKillop, 2013).

Beyond its association with ADHD symptoms, the G-allele of rs1800544 has been found to be related to alcohol dependence and tobacco smoking (Karahalil et al., 2008; Prestes et al., 2007). In addition, it has been discovered that carrying the G-allele was associated with a higher consumption of sweets (such as chocolate, candies and nougat) in a study with children and adolescents (Maestu et al., 2007) and also with an elevated stress response to cold and psychosocial stressors in adolescents and young adults (Kelsey et al., 2012). Finally, even though only few studies focused on the rs521674 and rs602618 polymorphisms, they have recently been associated with a positive family history of alcoholism (Clarke et al., 2012). These findings support the view that the minor alleles of these SNPs might impact addictive behaviors given that the common denominator of alcohol and tobacco dependence, stimulant addiction, and obesity is the propensity to favor immediate rewards at the expense of negative future consequence (MacKillop, 2013; Mazur, 1987). However, it should be noted that, beyond the shown *group*genotype* interaction on DD, we did not find an association between the investigated *ADRA2A* SNPs and cocaine use per se.

It is still uncertain whether the three investigated SNPs mapping to the promoter region of the *ADRA2A* gene have functional significance. However, theoretical transcription binding sites suggest that at least rs521674 and rs60218 create new transcription binding sites at the T and C polymorphisms, respectively (e.g., http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3). Additionally, the mRNA quantification in our present study provides an additional source of information. We found that cocaine users with high peripheral *ADRA2A* mRNA expression levels showed normal DD, while cocaine users with low mRNA expression levels revealed a strong DD endophenotype even though this effect was only a statistical trend (p=.07) in the post hoc comparison (Figure 2). Notably, the sample size for the mRNA expression analysis was much lower than for the SNP analysis (only 104 participants were eligible for the mRNA expression analysis) due to our strict mRNA quality control and, thus, the gene expression analysis was less well powered. However, post hoc power analyses revealed that group*genotype (f=0.22) and group*gene expression (f=0.23) interactions on DD displayed highly similar effect sizes.

Moreover, also the post hoc comparisons on DD in the cocaine group showed comparable effect sizes in the genotype (G vs. CG/GG: *d*=0.56) and gene expression analysis (high vs low: *d*=0.50). With this, also our mRNA findings corroborate that cocaine use moderates the effects of *ADRA2A* genotype on DD. Support for this assumption stems from two animal studies. On the one hand, chronic cocaine administration has been shown to desensitize or downregulate postsynaptic ADRA2As from 42 hours on up to eight days later in the rat brain (Baumann et al., 2004). On the other hand, the *ADRA2A* agonist guanfacine was able to reduce impulsivity in rhesus monkeys by increasing the proportion of choices of larger delayed rewards over smaller immediate rewards (Kim et al., 2012). Based on these two studies, we postulate that control individuals have unaffected ADRA2As, whereas in cocaine users, ADRA2As may have been desensitized or downregulated by chronic drug consumption. Under these circumstances, higher receptor mRNA expression may be capable of mimicking the beneficial effects of guanfacine resulting in normal DD.

In line with our findings, a recent review highlights the potential of guanfacine as a therapeutic agent to attenuate stress- and cue-induced craving in cocaine-dependent individuals (Fox and Sinha, 2014). The authors further propose that guanfacine may be an effective medication to reduce craving and relapse vulnerability in other forms of addiction as well, due to its ability to improve cognitive and emotional control over drug-seeking behavior (Fox and Sinha, 2014).

The present study has two limitations. First, because of the low frequency of the GG genotype of rs1800544 (8.8%) in combination with our limited sample size, we decided to contrast G-allele carriers against non-carriers. Even though the threefold genotype comparison (CC vs. CG vs. GG) revealed comparable results (Table S4 and Figure S1), we have chosen to rely on the two-group comparisons for our interpretations. Second, because of the cross-sectional analysis in this study, it is not possible to answer conclusively whether the *ADRA2A* genotype*cocaine use interaction on DD develops as a consequence of cocaine use or if DD together with *ADRA2A* SNPs predispose cocaine addiction, as there is evidence for both interpretations (Brody et al., 2014). Our recent longitudinal results suggest that – in

contrast to self-reported impulsivity and decision-making in the Iowa Gambling Task – DD is largely stable in cocaine users and does not change with decreased or increased use supporting the view that DD might be a predisposition and a potential endophenotype of cocaine addiction (Hulka et al., in press). However, the sample size of our longitudinal study is too small to investigate potential genotype effects on the course of DD. Thus, to draw definite conclusions, future studies should use longitudinal designs with larger samples in order to investigate the causal relationships between DD, cocaine use, and *ADRA2A* SNPs more in depth.

In conclusion, we presented evidence that relationship between the DD endophenotype and cocaine use is moderated by three SNPs of the *ADRA2A* gene as well as by *ADRA2A* gene expression suggesting that the norepinephrine system is involved in DD observed in cocaine users. As DD deficits may underlie addictive disorders in general, our results suggest that pharmacological compounds targeting *ADRA2A* (such as guanfacine) might be considered as option for symptom-specific treatment of DD in stimulant addiction.

Acknowledgments

We would like to thank Vera Guttenthaler and Miryame Hofmann for technical assistance. The study was supported by grants from the Swiss National Science Foundation (SNSF; grant No. PP00P1-123516/1 and PP00P1-146326/1) and the Olga Mayenfisch Foundation. None of the authors declared any conflict of interest.

Authors Contribution

BBQ was responsible for the study concept and design. LMA, MV and KHP contributed to the acquisition of data. ET and EG performed the mRNA analysis, RM performed the genotyping and MRB performed the toxicology analysis. MMH completed the data analysis and MMH and BBQ interpreted the findings. MMH and BBQ drafted the manuscript. CE and ES provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

References

American Psychiatric Association (1994) *Diagnostic and Statistical Manual of Mental Disorders (4th edn) (DSM-IV)*. APA.

Arnsten AF, Steere JC, Hunt RD (1996) The contribution of alpha 2-noradrenergic mechanisms of prefrontal cortical cognitive function. Potential significance for attention-deficit hyperactivity disorder. Arch Gen Psychiatry 53:448-455.

Bari A, Robbins TW (2013) Noradrenergic versus dopaminergic modulation of impulsivity, attention and monitoring behaviour in rats performing the stop-signal task: possible relevance to ADHD. Psychopharmacology (Berl) 230:89-111.

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263-265.

Baumann MH, Milchanowski AB, Rothman RB (2004) Evidence for alterations in alpha2-adrenergic receptor sensitivity in rats exposed to repeated cocaine administration. Neuroscience 125:683-690.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J (1961) An inventory for measuring depression. Arch Gen Psychiatry 4:561-571.

Bickel WK, Landes RD, Christensen DR, Jackson L, Jones BA, Kurth-Nelson Z, Redish AD (2011) Single- and cross-commodity discounting among cocaine addicts: the commodity and its temporal location determine discounting rate. Psychopharmacology (Berl) 217:177-187.

Brody GH, Yu T, MacKillop J, Miller GE, Chen E, Obasi EM, Beach SR (2014) Catecholamine levels and delay discounting forecast drug use among African American youths. Addiction 109:1112-1118.

Buffalari DM, Baldwin CK, See RE (2012) Treatment of cocaine withdrawal anxiety with guanfacine: relationships to cocaine intake and reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 223:179-190.

Clark AJ, Butcher SP, Winn P (1991) Evidence for functional separation of alpha-1 and alpha-2 noradrenaline receptors by pre-synaptic terminal re-uptake mechanisms. Psychopharmacology (Berl) 103:366-374.

Clarke TK, Dempster E, Docherty SJ, Desrivieres S, Lourdsamy A, Wodarz N, Ridinger M, Maier W, Rietschel M, Schumann G (2012) Multiple polymorphisms in genes of the adrenergic stress system confer vulnerability to alcohol abuse. Addict Biol 17:202-208.

Coffey SF, Gudleski GD, Saladin ME, Brady KT (2003) Impulsivity and rapid discounting of delayed hypothetical rewards in cocaine-dependent individuals. Exp Clin Psychopharmacol 11:18-25.

Cummins TD, Jacoby O, Hawi Z, Nandam LS, Byrne MA, Kim BN, Wagner J, Chambers CD, Bellgrove MA (2014) Alpha-2A adrenergic receptor gene variants are associated with increased intra-individual variability in response time. Mol Psychiatry 19:1031-1036.

de Cerqueira CC, Polina ER, Contini V, Marques FZ, Grevet EH, Salgado CA, da Silva PO, Picon FA, Belmonte-de-Abreu P, Bau CH (2011) ADRA2A polymorphisms and ADHD in adults: possible mediating effect of personality. Psychiatry Res 186:345-350.

Fitzgerald PJ (2013) Elevated norepinephrine may be an etiological factor in a wide range of diseases: age-related macular degeneration, systemic lupus erythematosus, atrial fibrillation, metabolic syndrome. Med Hypotheses 80:558-563.

Fox H, Sinha R (2014) The role of guanfacine as a therapeutic agent to address stress-related pathophysiology in cocaine-dependent individuals. Adv Pharmacol 69:217-265.

Fox HC, Seo D, Tuit K, Hansen J, Kimmerling A, Morgan PT, Sinha R (2012) Guanfacine effects on stress, drug craving and prefrontal activation in cocaine dependent individuals: preliminary findings. J Psychopharmacol 26:958-972.

Gizer IR, Ficks C, Waldman ID (2009) Candidate gene studies of ADHD: a meta-analytic review. Hum Genet 126:51-90.

Grunblatt E, Bartl J, Zehetmayer S, Ringel TM, Bauer P, Riederer P, Jacob CP (2009) Gene expression as peripheral biomarkers for sporadic Alzheimer's disease. J Alzheimers Dis 16:627-634.

Heil SH, Johnson MW, Higgins ST, Bickel WK (2006) Delay discounting in currently using and currently abstinent cocaine-dependent outpatients and non-drug-using matched controls. Addict Behav 31:1290-1294.

Hochberg Y (1988) A sharper Bonferroni procedure for multiple tests of significance. Biometrika 75:3.

Hulka LM, Eisenegger C, Preller KH, Vonmoos M, Jenni D, Bendrick K, Baumgartner MR, Seifritz E, Quednow BB (2014) Altered social and non-social decision-making in recreational and dependent cocaine users. Psychol Med 44:1015-1028.

Hulka LM, Vonmoos M, Preller KH, Baumgartner MR, Seifritz E, Gamma A, Quednow BB (in press) Changes in cocaine consumption are associated with fluctuations in self-reported impulsivity and gambling decision-making. Psychol Med.

Karahalil B, Coskun E, Emerce E (2008) ADRA2A polymorphism and smoking in a Turkish population. Toxicol Ind Health 24:171-176.

Kelsey RM, Alpert BS, Dahmer MK, Krushkal J, Quasney MW (2012) Alpha-adrenergic receptor gene polymorphisms and cardiovascular reactivity to stress in Black adolescents and young adults. Psychophysiology 49:401-412.

Kim S, Bobeica I, Gamo NJ, Arnsten AF, Lee D (2012) Effects of alpha-2A adrenergic receptor agonist on time and risk preference in primates. Psychopharmacology (Berl) 219:363-375.

Kirby KN, Petry NM (2004) Heroin and cocaine abusers have higher discount rates for delayed rewards than alcoholics or non-drug-using controls. Addiction 99:461-471.

Koob GF (2009) Dynamics of neuronal circuits in addiction: reward, antireward, and emotional memory. Pharmacopsychiatry 42 Suppl 1:S32-41.

Lehrl S (1999) *Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B)*. 4th ed. Hogrefe: Göttingen.

Levy MN, Blattberg B (1978) The influence of cocaine and desipramine on the cardiac responses to exogenous and endogenous norepinephrine. Eur J Pharmacol 48:37-49.

Li T, Zhu X, Wu X, Li J, Pan L, Li P, Xin Z, Gu HF, Liu Y (2012) Evaluation of the association between the ADRA2A genetic polymorphisms and type 2 diabetes in a Chinese Han population. Genet Test Mol Biomarkers 16:1424-1427.

MacKillop J (2013) Integrating behavioral economics and behavioral genetics: delayed reward discounting as an endophenotype for addictive disorders. J Exp Anal Behav 99:14-31.

Maestu J, Villa I, Parik J, Paaver M, Merenakk L, Eensoo D, Harro M, Harro J (2007) Human adrenergic alpha 2A receptor C-1291G polymorphism leads to higher consumption of sweet food products. Mol Psychiatry 12:520-521.

Mazur J (1987) *An adjusting procedure for delayed reinforcement.* Erlbaum: New Jersey.

Mischel W, Ayduk O, Berman MG, Casey BJ, Gotlib IH, Jonides J, Kross E, Teslovich T, Wilson NL, Zayas V, Shoda Y (2011) 'Willpower' over the life span: decomposing self-regulation. Soc Cogn Affect Neurosci 6:252-256.

Moffitt TE, Arseneault L, Belsky D, Dickson N, Hancox RJ, Harrington H, Houts R, Poulton R, Roberts BW, Ross S, Sears MR, Thomson WM, Caspi A (2011) A gradient of childhood self-control predicts health, wealth, and public safety. Proc Natl Acad Sci U S A 108:2693-2698.

Noreika V, Falter CM, Rubia K (2013) Timing deficits in attention-deficit/hyperactivity disorder (ADHD): evidence from neurocognitive and neuroimaging studies. Neuropsychologia 51:235-266.

O'Neill B, Tilley MR, Gu HH (2013) Cocaine produces conditioned place aversion in mice with a cocaine-insensitive dopamine transporter. Genes Brain Behav 12:34-38.

Park L, Nigg JT, Waldman ID, Nummy KA, Huang-Pollock C, Rappley M, Friderici KH (2005) Association and linkage of alpha-2A adrenergic receptor gene polymorphisms with childhood ADHD. Mol Psychiatry 10:572-580.

Perez de Los Cobos J, Sinol N, Puerta C, Cantillano V, Lopez Zurita C, Trujols J (2011) Features and prevalence of patients with probable adult attention deficit hyperactivity disorder who request treatment for cocaine use disorders. Psychiatry Res 185:205-210.

Preller KH, Hulka LM, Vonmoos M, Jenni D, Baumgartner MR, Seifritz E, Dziobek I, Quednow BB (2014) Impaired emotional empathy and related social network deficits in cocaine users. Addict Biol 19:452-466.

Prestes AP, Marques FZ, Hutz MH, Roman T, Bau CH (2007) Tobacco smoking and the ADRA2A C-1291G polymorphism. J Neural Transm 114:1503-1506.

Quednow BB, Kuhn KU, Hoenig K, Maier W, Wagner M (2004) Prepulse inhibition and habituation of acoustic startle response in male MDMA ('ecstasy') users, cannabis users, and healthy controls. Neuropsychopharmacology 29:982-990.

Robinson ES, Eagle DM, Mar AC, Bari A, Banerjee G, Jiang X, Dalley JW, Robbins TW (2008) Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. Neuropsychopharmacology 33:1028-1037.

Roman T, Polanczyk GV, Zeni C, Genro JP, Rohde LA, Hutz MH (2006) Further evidence of the involvement of alpha-2A-adrenergic receptor gene (ADRA2A) in inattentive dimensional scores of attention-deficit/hyperactivity disorder. Mol Psychiatry 11:8-10.

Roman T, Schmitz M, Polanczyk GV, Eizirik M, Rohde LA, Hutz MH (2003) Is the alpha-2A adrenergic receptor gene (ADRA2A) associated with attention-deficit/hyperactivity disorder? Am J Med Genet B Neuropsychiatr Genet 120B:116-120.

Rosler M, Retz W, Retz-Junginger P, Thome J, Supprian T, Nissen T, Stieglitz RD, Blocher D, Hengesch G, Trott GE (2004) [Tools for the diagnosis of attention-deficit/hyperactivity disorder in adults. Self-rating behaviour questionnaire and diagnostic checklist]. Nervenarzt 75:888-895.

Rounsaville BJ (2004) Treatment of cocaine dependence and depression. Biol Psychiatry 56:803-809.

Schmitz M, Denardin D, Silva TL, Pianca T, Roman T, Hutz MH, Faraone SV, Rohde LA (2006) Association between alpha-2a-adrenergic receptor gene and ADHD inattentive type. Biol Psychiatry 60:1028-1033.

Stevenson J, Langley K, Pay H, Payton A, Worthington J, Ollier W, Thapar A (2005) Attention deficit hyperactivity disorder with reading disabilities: preliminary genetic findings on the involvement of the ADRA2A gene. J Child Psychol Psychiatry 46:1081-1088.

Urcelay GP, Dalley JW (2012) Linking ADHD, impulsivity, and drug abuse: a neuropsychological perspective. Curr Top Behav Neurosci 9:173-197.

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3:RESEARCH0034.

Vonmoos M, Hulka LM, Preller KH, Jenni D, Baumgartner MR, Stohler R, Bolla KI, Quednow BB (2013) Cognitive dysfunctions in recreational and dependent cocaine users: role of attention-deficit hyperactivity disorder, craving and early age at onset. Br J Psychiatry 203:35-43.

Vonmoos M, Hulka LM, Preller KH, Minder F, Baumgartner MR, Quednow BB (2014) Cognitive impairment in cocaine users is drug-induced but partially reversible: evidence from a longitudinal study. Neuropsychopharmacology 39:2200-2210.

Washio Y, Higgins ST, Heil SH, McKerchar TL, Badger GJ, Skelly JM, Dantona RL (2011) Delay discounting is associated with treatment response among cocaine-dependent outpatients. Exp Clin Psychopharmacol 19:243-248.

Wittchen HU, Wunderlich U, Gruschwitz S, Zaudig M (1997) *SKID-I. Strukturiertes Klinisches Interview für DSM-IV Achse I: Psychische Störungen. [SCID-I. Structured Clinical Interview for DSM-IV Axis I Disorders]*. Hogrefe.

Figure Legends

Figure 1 Mean delay discounting (DD) across reward magnitudes for rs1800544 with significant (p<0.01) *group*genotype* interactions (mean and *SE*, z-transformed and corrected for *age* and *verbal IQ*). Higher scores depict decreased discounting, whereas lower scores show increased discounting. Significant post hoc t-test on genotype (CC vs. CG-GG) in cocaine users: **p<.01.

Figure 2 Mean delay discounting (DD) across reward magnitudes in cocaine users and controls stratified for high vs. low *ADRA2A* mRNA expression levels (mean and *SE*, z-transformed and corrected for *age* and *verbal IQ*). Higher scores depict decreased discounting, whereas lower scores show increased discounting. Near-significant post hoc t-test on genotype (CC vs. CG-GG) in cocaine users: $^{(*)}p$=.07.

Table 1 Demographic data of healthy controls and cocaine users (with exception of sex, means and *SD* are shown).

Demographic Data	**Controls (*n*=94)**	**Cocaine users (*n*=129)**	***t-test/X²***	***df***	***p***
Age, mean (*SD*)	30.0 (8.8)	30.0 (8.8)	-0.00	221	0.968
Sex (male / female)	68 / 26	94 / 35	0.0	1	0.524
Years of Education, mean (*SD*)	10.7 (1.8)	10.2 (1.7)	2.1	221	**0.038**
Verbal IQ, mean (*SD*)	107.1 (12.1)	102.4 (10.5)	3.0	183.1	**0.003**
BDI Score, mean (*SD*)	4.2 (4.0)	8.5 (7.0)	-5.8	210.2	**0.000**
ADHD-SR Score, mean (*SD*)	7.9 (5.0)	14.0 (9.3)	-6.2	204.0	**0.000**

ADHD-SR: Attention Deficit Hyperactivity Disorder-Self Rating Scale, BDI: Beck Depression Inventory, IQ: intelligence quotient

Table 2 Analyses of covariance for delay discounting (DD) across reward magnitudes (corrected for *age* and *verbal IQ*).

	***ADRA2A* (rs1800544)**				
	F	*df*	p_{ncor}	ηp^2	p_{cor}
DD (across all rewards)					
Group	3.32	1	.070	.015	
Genotype	0.92	1	.338	.004	
Group*Genotype	10.05	1	**.002**	.045	**.004**
DD (small rewards)					
Group	7.89	1	**.005**	.035	**.010**
Genotype	2.14	1	.145	.010	
Group*Genotype	12.49	1	**.001**	.055	**.002**
DD (medium rewards)					
Group	8.05	1	**.005**	.036	**.005**
Genotype	2.07	1	.152	.010	
Group*Genotype	7.27	1	**.008**	.033	**.008**
DD (large rewards)					
Group	6.55	1	**.011**	.030	**.033**
Genotype	1.96	1	.163	.009	
Group*Genotype	6.98	1	**.009**	.031	**.027**

DD = delay discounting, p_{ncor}: uncorrected *p*-values, p_{cor}: p-values corrected for multiple comparisons

Figure 1

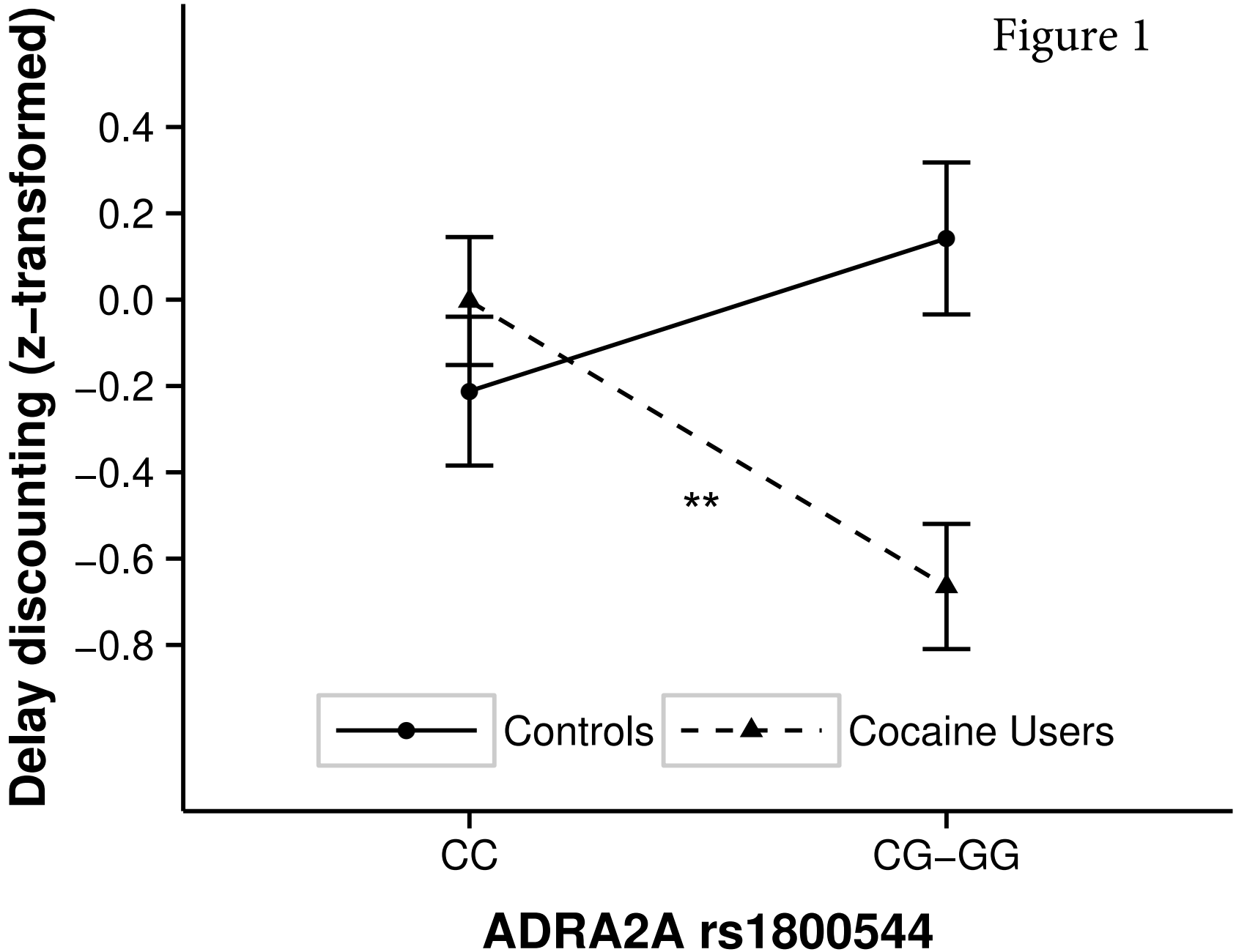
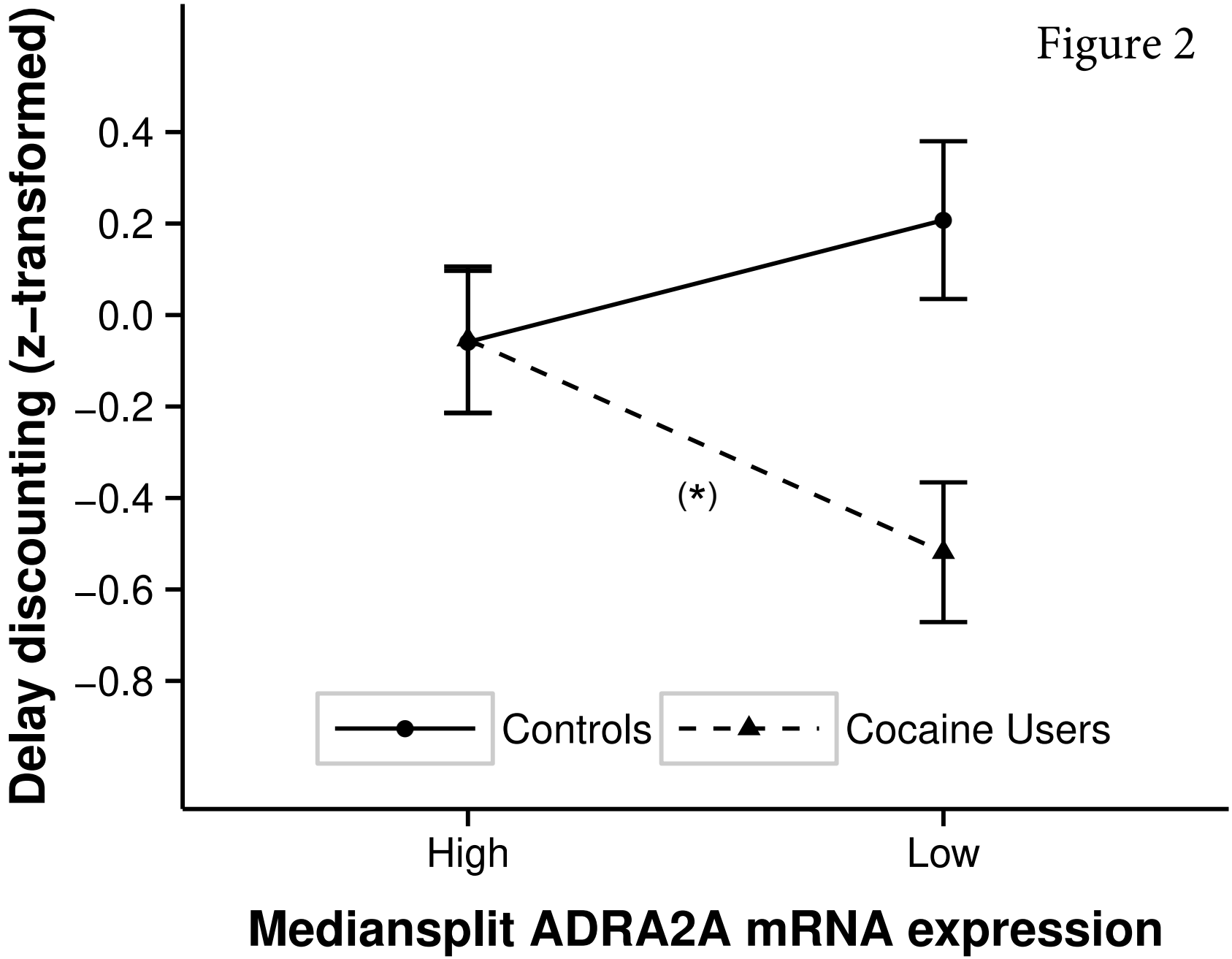

Delay discounting (z–transformed)
0.4
0.2
0.0
–0.2
–0.4
–0.6
–0.8
**
Controls
Cocaine Users
CC
CG–GG
ADRA2A rs1800544

Figure 2


Delay discounting (z–transformed)
0.4
0.2
0.0
–0.2
–0.4
–0.6
–0.8
(*)
Controls
Cocaine Users
High
Low
Mediansplit ADRA2A mRNA expression

Supplementary Information

α_{2A}-adrenergic receptor polymorphisms and mRNA expression levels are associated with delay discounting in cocaine users

Michael M Havranek, Lea M Hulka, Eve Tasiudi, Christoph Eisenegger, Matthias Vonmoos, Katrin H Preller, Rainald Mössner, Markus R Baumgartner, Erich Seifritz, Edna Grünblatt, Boris B Quednow

Methods

Genotyping: DNA for the SNP genotyping was extracted either from EDTA anticoagulated blood samples or from immortalized lymphoblastoids cell cultures after transforming the lymphocytes with Epstein-Barr virus. The isolation of the DNA followed the QIAGEN protocol for the Blood & Cell Culture DNA Isolation Maxi Kit (QIAGEN, Hilden, Germany). For PCR, we added 5µl of buffer containing the Universal PCR MasterMix (No AmpErase UNG) and the SNP Genotyping Assay (both provided by Applied Biosystems, Foster City, CA, USA) to 12.5ng air-dried DNA. PCR was performed according to the SNP Genotyping protocol supplied by Applied Biosystems. Both alleles were scored in a single well by measuring the fluorescence at the end of the PCR using a Tecan Ultra 384 reader (Tecan, Crailsheim, Germany). Excitation- and emission-wavelengths were 485 and 535 nm for the FAM-labeled probes and 535 and 590 nm for the VIC-labeled probes, respectively.

Table S1 Substance use.

Substance use	**Controls (*n*=94)**	**Cocaine users (*n*=129)**
Weekly[a] nicotine use, cigarettes, mean (*SD*)	62.0 (66.5)	90.4 (75.0)
Weekly[a] alcohol dose, g, mean (*SD*)	123.6 (135.9)	182.8 (194.2)
Weekly[a] cannabis use, g, mean (*SD*)	0.6 (1.4)	1.0 (2.8)
Weekly[a] MDMA use, g, mean (*SD*)	0.0 (0.0)	0.2 (0.9)
Weekly[a] amphetamine use, g, mean (*SD*)	0.0 (0.0)	0.1 (0.2)
Weekly[a] cocaine dose, g, mean (*SD*)	0.0 (0.0)	2.4 (5.0)
Years of cocaine use, mean (*SD*)	0.0 (0.0)	7.0 (5.5)
Cocaine concentration in hair, pg/mg, mean (*SD*)	0.0 (0.0)	7989 (18722)
Cumulative cocaine dose, g, mean (*SD*)	0.0 (0.0)	2143 (5798)
Self-reported cocaine abstinence duration, hours, mean (SD)	-	650.3 (861.5)
Recent cocaine use, urine samples, positive/negative/missing	0/94/0	26/102/1

[a]Weekly use is referring to the last six months, MDMA = 3,4-Methylendioxy-N-methylamphetamine

Table S2 Genotype frequencies of all three investigated polymorphisms in total sample, in controls and in cocaine users.

Polymorphism	Genotype frequencies (n/%)		Deviation from HWE	in controls (n/%)	in users (n/%)	Association with group
ADRA2A (rs1800544)	CC	110/49.8		47/51.1	63/48.8	
	CG	91/41.2	$X^2(1) = .002$, $p = .963$	37/40.2	54/41.9	$X^2(1) = .11$, $p = .946$
	GG	20/9		8/8.7	12/9.3	
ADRA2A (rs521674)	AA	109/49.3		47/51.1	62/48.1	
	AT	92/41.6	$X^2(1) = .002$, $p = .963$	37/40.2	55/42.6	$X^2(1) = .20$, $p = .906$
	TT	20/9		8/8.7	12/9.3	
ADRA2A (rs602618)	AA	104/47.3		45/48.9	59/46.1	
	AC	95/43.2	$X^2(1) = .055$, $p = .814$	39/42.4	56/43.8	$X^2(1) = .23$, $p = .890$
	CC	21/9.5		8/8.7	13/10.2	

HWE = Hardy-Weinberg Equilibrium

Table S3 Analysis of variance for cocaine use across genotype groups.

Cocaine use	Genotype	Cocaine conc. in hair, pg/mg, mean (*SD*)	*F*	*df*, df_{err}	*p*
ADRA2A (rs1800544)	CC	9235 (21598)	0.341	2, 123	0.711
	CG	7217 (16656)			
	GG	4899 (9382)			
ADRA2A (rs521674)	AA	8944 (21654)	0.252	2, 123	0.778
	AT	7590 (16718)			
	TT	4899 (9382)			
ADRA2A (rs602618)	AA	9730 (22249)	0.540	2, 122	0.584
	AC	6993 (16380)			
	CC	4536 (9078)			

Cocaine use	Genotype	Cumulative cocaine dose, g, mean (*SD*)	*F*	*df*, df_{err}	*p*
ADRA2A (rs1800544)	CC	1285 (3021)	2.008	2, 125	0.139
	CG	2563 (6345)			
	GG	4689 (11469)			
ADRA2A (rs521674)	AA	1280 (3046)	1.996	2, 125	0.140
	AT	2546 (6287)			
	TT	4689 (11469)			
ADRA2A (rs602618)	AA	1346 (3086)	1.610	2, 124	0.204
	AC	2517 (6295)			
	CC	4341 (11053)			

Table S4 Analyses of covariance for delay discounting across reward magnitudes (unpooled genotypes: CC vs. CG vs. GG, corrected for *age* and *verbal IQ*).

	ADRA2A (rs1800544)				
	F	*df*	p_{ncor}	ηp^2	p_{cor}
DD (across all rewards)					
Group	1.21	1	.272	.006	
Genotype	0.62	2	.540	.006	
Group*Genotype	6.47	2	**.002**	.057	**.004**
DD (small rewards)					
Group	2.05	1	.154	.010	
Genotype	1.12	2	.327	.010	
Group*Genotype	9.63	2	**.000**	.083	**.000**
DD (medium rewards)					
Group	3.84	1	**.050**	.018	
Genotype	1.07	2	.345	.010	
Group*Genotype	4.52	2	**.012**	.041	**.024**
DD (large rewards)					
Group	4.06	1	**.045**	.019	.135
Genotype	1.00	2	.371	.009	
Group*Genotype	3.85	2	**.023**	.035	.069

DD = delay discounting, p_{ncor}: uncorrected *p*-values, p_{cor}: *p*-values corrected for multiple comparisons

Table S5 Analyses of covariance for delay discounting across reward magnitudes (haplotype analysis: CC-AA-AA vs. CG/GG-AT/TT-AC/CC, ten participants with rare allele combinations were excluded, corrected for *age* and *verbal IQ*).

	ADRA2A Haplotype			
	F	*df*	*p*	ηp^2
DD (across all rewards)				
Group	2.46	1	.118	.012
Genotype	0.80	1	.373	.004
Group*Genotype	10.30	1	**.002**	.047
DD (small rewards)				
Group	6.19	1	**.014**	.029
Genotype	1.96	1	.163	.009
Group*Genotype	12.77	1	**.000**	.058
DD (medium rewards)				
Group	6.38	1	**.012**	.030
Genotype	2.29	1	.132	.011
Group*Genotype	7.78	1	**.006**	.036
DD (large rewards)				
Group	5.58	1	**.019**	.026
Genotype	1.77	1	.185	.008
Group*Genotype	6.56	1	**.011**	.031

DD = delay discounting

Table S6 Analysis of variance for mRNA expression levels in controls and cocaine users and across genotype groups (means and *SD*).

		Peripheral ADRA2A mRNA expression, mean (*SD*)					
mRNA expression	**Genotype**	**in controls**	**in cocaine users**		***F***	***df, df*$_{err}$**	***p***
ADRA2A (rs1800544)	CC	1.30 (.74)	1.33 (1.2)	**Factor group**	0.543	1, 97	0.463
	CG	1.31 (.88)	1.27 (.65)	**Factor genotype**	0.814	2, 97	0.446
	GG	2.10 (1.3)	1.48 (1.8)	**Group*genotype**	0.345	2, 97	0.709
ADRA2A (rs521674)	AA	1.30 (.74)	1.37 (1.2)	**Factor group**	0.528	1, 97	0.469
	AT	1.31 (.88)	1.25 (.65)	**Factor genotype**	0.838	2, 97	0.436
	TT	2.10 (1.3)	1.48 (1.8)	**Group*genotype**	0.404	2, 97	0.669
ADRA2A (rs602618)	AA	1.36 (.72)	1.26 (1.1)	**Factor group**	0.853	1, 97	0.358
	AC	1.19 (.85)	1.28 (.66)	**Factor genotype**	0.984	2, 97	0.378
	CC	2.10 (1.3)	1.40 (1.5)	**Group*genotype**	0.616	2, 97	0.542

Figure S1

Fig. S1 Linkage disequilibrium (LD) plot of the three polymorphisms (rs1800544, rs521674, and rs602618) with SNPs in near perfect LD depicted in red (rs1800544/rs521674: D'=1.0, LOD=92.24, r2=0.99; rs1800544/rs602618: D'=0.98, LOD=79.62, r2=0.92; rs521674/rs602618: D'=0.97, LOD=77.66, r2=0.91).

Figure S2

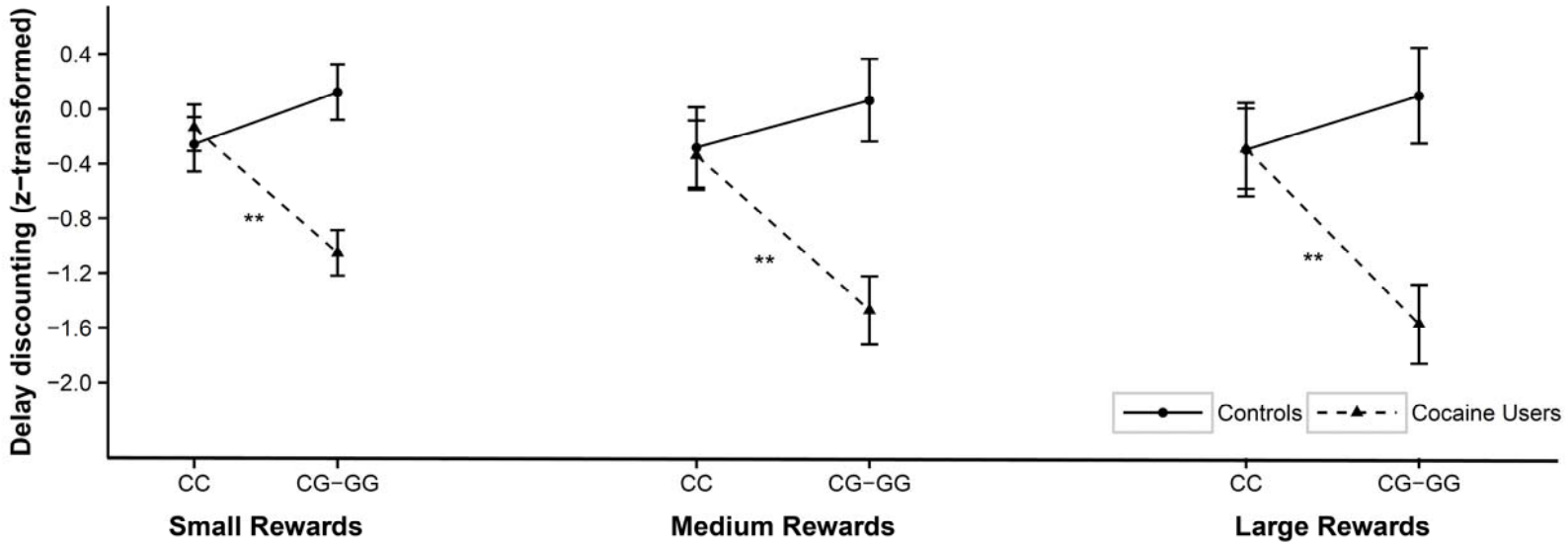


Fig. S2 Mean delay discounting (DD) of small, medium, and large reward magnitudes for rs1800544 with significant (p<0.01) *group*genotype* interactions (mean and *SE*, z-transformed and corrected for *age* and *verbal IQ*). Higher scores depict decreased discounting, whereas lower scores show increased discounting. Significant post hoc t-tests on genotype (CC vs. CG-GG) in cocaine users: **p<.01.

Figure S3

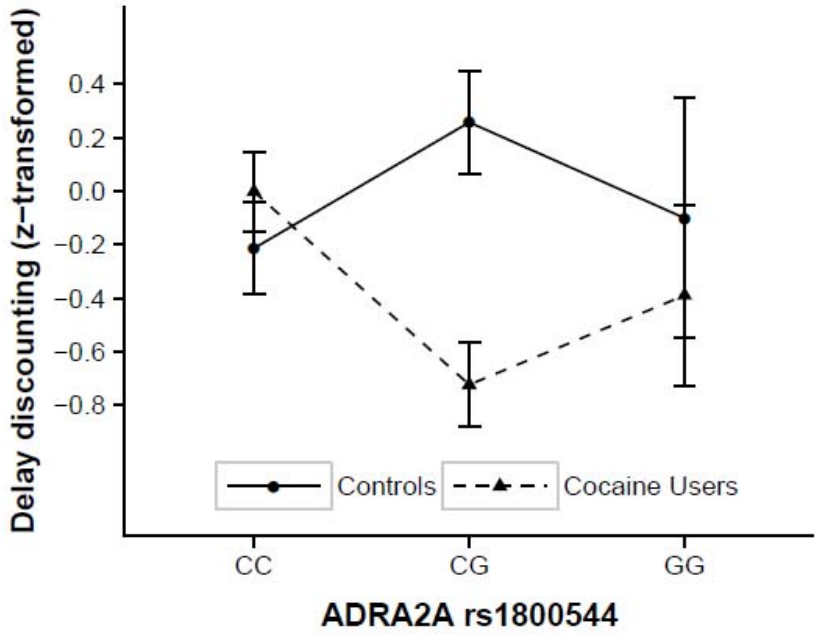


Fig. S3 Mean delay discounting scores across reward magnitudes (unpooled genotypes: CC vs. CG vs. GG) with significant *group*genotype* interactions (mean and *SE*, z-transformed and corrected for *age* and *verbal IQ,* one subject has been excluded). Higher scores depict decreased discounting, whereas lower scores show increased discounting.

Figure S4

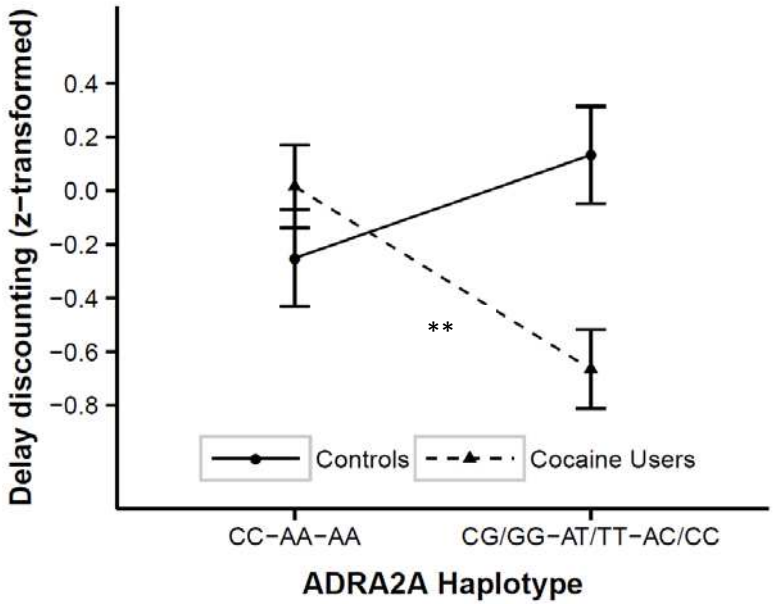


Fig. S4 Mean delay discounting scores across reward magnitudes (haplotype analysis: CC-AA-AA vs. CG/GG-AT/TT-AC/CC,) with significant *group*genotype* interactions (mean and *SE*, z-transformed and corrected for *age* and *verbal IQ,* ten participants with rare allele combinations were excluded). Higher scores depict decreased discounting, whereas lower scores show increased discounting. Significant post hoc t-test on genotype (CC vs. CG-GG) in cocaine users: **p<.01.